SCIENTIFIC MEMOIRS

BY

MEDICAL OFFICERS OF THE ARMY OF INDIA.

EDITED BY

B. SIMPSON, ESQ., M.D.,

SURGEON-GENERAL WITH THE GOVERNMENT OF INDIA.

PART I.

1884.

CALCUTTA:
PRINTED BY THE SUPERINTENDENT OF GOVERNMENT PRINTING, INDIA.
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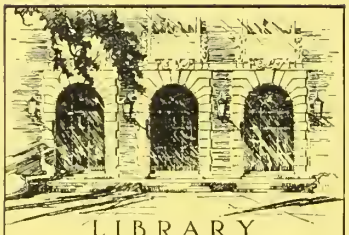

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On the relation of Cholera to Schizomycete organisms.

BY

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The following paper dealing with certain points in the vexed question of the relation of cholera to schizomycete organisms, and specially of its relation to so-called "comma bacilli," contains an account of the results attained in a series of investigations which were conducted in Calcutta during the current year and mainly during the hot and rainy seasons.

In order so far as possible to avoid laying undue importance on all matter of a purely controversial nature, attention has been almost entirely confined to an account of the observations themselves, only occasional and casual reference being made to the data which have been already published by other observers. This has not been done from any want of recognition of their value or from any wish to depreciate the work of others, but solely because the statements which have been made in regard to many points are so conflicting and irreconcilable in their nature that, were any special reference made to any apparent confirmation or contradiction of any of them, an appearance of partisanship might be imparted to what ought to be a mere statement of results.

The entire series of observations was specially related to the following points :—

I.—The association of curved schizomycete organisms with materials of choleraic nature;

II.—The presence of similar bodies in other media;

III.—The effects of variations in the nature of the medium in which they are developed on the curved schizomycetes occurring in association with choleraic materials.

Due to the time of year at which the cultivations of choleraic and other materials were principally carried on—a time during which the conditions of temperature were such as to preclude the use of common gelatine solid media—they were all conducted in agar-agar jelly or in fluids of various kinds. The agar-agar jelly was prepared according to the formula for the common gelatine

basis in Professor Johne's account of the methods followed in Dr. Koch's laboratory during the conduct of the courses of bacteriological teaching carried out there in reference to the diagnosis of cholera by means of the results of cultivations of comma bacilli.[1] The amount of agar-agar employed was, however, considerably less than that of the gelatine in the formula, as it was ascertained that about 1·5 per cent. was sufficient to secure a basis which would remain solid in the temperatures to which it was ordinarily exposed. As the temperature was fairly uniform and constantly high, comparatively little use was made of the incubator, the cultivations being, as a rule, conducted at the ordinary temperature of the laboratory.

In some cases the substratum was neutralised or rendered faintly alkaline by means of the addition of solutions of carbonate of soda; in others its normal acidity was reduced by similar means; and in still others it was allowed to retain its natural reaction.

This substratum served as a basis for plate and tube cultivations, and in certain of the latter was so far diluted as to cause it to remain permanently fluid. In other cases acid, neutral or alkaline broth, was employed in tube, cultivations.

Microscopical preparations of the materials employed in the cultivations and of the growths connected with them, were made by the usual methods of preparing dried films on cover-glasses, staining these with different aniline dyes and washing out the excess of color in spirit. A number of dyes were occasionally employed, but in the majority of instances Spiller's purple and fuchsin were those which were used. Where sections of tissues were prepared Williams' freezing microtome was made use of, as it was found that in an atmosphere so hot and humid as that of Calcutta is during the hot and rainy seasons instruments depending on the action of ether did not give satisfactory results.

The microscopes employed in the examination of specimens consisted of a Zeiss fitted with an Abbe's condenser and lenses B, C, D, and $\frac{1}{12}$ oil immersion, and one of Beck's "Pathological" microscopes with lenses $\frac{4}{10}$, $\frac{1}{5}$, and $\frac{1}{8}$ of the same makers, and $\frac{1}{16}$ and $\frac{1}{20}$ oil immersion lenses by Powell and Lealand.

Each of the special subjects of investigation will now be considered in succession.

I.—The association of curved Schizomycete organisms with materials of choleraic nature.

A.—Results of Examinations of recent Choleraic Materials.

1. Alvine evacuations.

The materials of this nature which were examined were obtained from the majority of the patients who were admitted into the wards of the Medical College Hospital during the current year as cases of cholera. By far the greater number of these occurred during the months of March, April, and May, there

[1] Ueber die Koch'schen Reinculturen und die Cholera Bacillen.—Von Dr. Albert Johne. Leipzig, 1885.

having been hardly any choleraic admissions during the latter part of the cold weather and the normal fall in prevalence of the disease having taken place towards the close of the hot weather.

The following table shows the total number of cases from which materials of any kind were obtained during each month from January to September :—

January	0
February	3
March	9
April	11
May	6
June	3
July	3
August	1
September	1
	37

It is curious to observe how even in such a limited number of cases the monthly distribution agrees with the data which we possess regarding the general normal seasonal prevalence of the disease.

It will be seen that the total number of cases of supposed choleraic nature from which materials of any kind were obtained was 37. One of them, however, that occurring in August, was subsequently ascertained not to be of a choleraic nature, and in regard to one or two of the others, a suspicion that the same was the case certainly exists. These cases will receive special notice further on.

Samples of fresh alvine evacuations were obtained in 28 cases. In 26 of these curved schizomycetes were recognisable. In two of them none could be detected. In both of these the flocculi which were prepared for examination appeared to be composed of almost pure mucus containing numerous blood corpuscles and curiously polynucleate mucus corpuscles but hardly any schizomycete organisms of any kind.

The numbers as well as the characters of the commas present in the remaining 26 cases varied very greatly. In ten cases they were very abundant, and in several of these the material approached a pure cultivation in its characters. In seven of the ten cases the commas were characteristic in appearance resembling those developed in artificial cultivations. In two they were characterised by their length, slenderness, and slight degree of curvature. In one they were quite unlike those occurring in any other instance, consisting of very large, relatively thick, strongly curved bodies. In the remaining 16 cases they were present in varying but relatively small numbers and of characteristic appearance.

2. Intestinal contents.

Specimens of Iliac mucus were examined in five cases.

In all cases commas were present. In four cases very few were present

and straight bacilli of various kinds abounded. In the specimens derived from the remaining case the conditions were reversed commas being present in very great numbers and few specimens of any other form being associated with them.

In connection with this subject it may be well to note that in none of the cases from which these materials were obtained was there any conspicuous signs of congestion of the intestinal mucous membrane, and that in two of them not the faintest evidence of any such condition could be detected.

3. Blood.

Specimens of blood were obtained from eight cases—in four during life and in the others during the course of *post mortem* examination. The samples in the first class of cases were taken from the tips of the fingers which had been previously carefully cleansed with spirit and solution of corrosive sublimate. The needles employed for puncture were heated to redness immediately previous to being employed and the blood was received in freshly drawn capillary pipettes.

In those cases where the blood was obtained after death the samples were taken by means of passing the extremities of freshly drawn closed capillary pipettes through the walls of the right auricle and subsequently breaking of the points within the cavity.

Numerous preparations were made from the materials thus obtained, but in no instance could the presence of schizomycete organisms of any kind be detected in them.

B.—Results of Cultivations of Choleraic Materials.

1. Cultivations of alvine evacuations.

Plate cultivations of this nature were carried out in 18 cases and in 13 of these colonies of characteristic commas made their appearance. In the remaining five cases no commas could be detected. In one of these cases the substratum of the cultivation was of acid reaction, in one the material from which the cultivation was derived was certainly not of truly choleraic nature, and in two others it appeared to be very probable that the same was the case, the reaction of the evacuation being of a strongly acid character in one of them, and the microscopical features of the other being very exceptional and unlike those ordinarily present in choleraic materials. In one case only in which there could be no reasonable doubt as to the choleraic nature of the disease, and in which the cultivation was conducted in an alkaline substratum did commas fail to appear. The proportions which the colonies of commas bore to those of other forms of schizomycete organisms varied very greatly in different instances. In some cultivations only one or two colonies could be detected among numerous others of different nature; in other cases they were present in large numbers; and in one, at all events, an almost pure cultivation of commas occurred.

2. Cultivations of materials obtained from the intestinal cavity after death.

Cultivations of Iliac mucus were carried out in four cases.

In three instances crops of characteristic commas were obtained. In two of them colonies of commas were developed in large numbers. In the third case they were very scanty, whilst colonies of minute, straight bacilli abounded.

In one case no commas could be detected. In this the substratum of the cultivation was of an acid reaction, but this condition was also present in one of the cultivations which yielded an abundant crop of commas.

3. Cultivations of blood.

Eighteen distinct tube cultivations of blood obtained from the eight cases of cholera previously alluded to were carried out, three or four punctures being made in each tube. In 16 tubes no development of any kind occurred. In the remaining two, which both belonged to the same case, a growth of what ultimately proved to be Micrococcus luteus took place along the lines of puncture and gradually spread thence over the surface.

4. Cultivations of other choleraic materials.

In three cases tube cultivations of juice from the liver and kidney of cases of cholera were attempted. The material for cultivation was obtained by means of carefully cleansing the surface of the organs with spirit and solution of corrosive sublimate, introducing the extremity of a freshly drawn, closed capillary pipette through the cleaned area into the subjacent tissues, and then breaking the tip and applying pressure around it. In two cases the cultivations were not attended with any result. In the remaining case the cultivation of kidney juice behaved in the same fashion, while schizomycete organisms of various forms appeared in that of liver juice. These, however, were unquestionably of salivary origin and connected with the fact that strong suction had been applied to the distal extremity of the pipette in the attempt to obtain a sufficient supply of juice.

Certain points in connection with the cultivations of materials of intestinal origin call for somewhat closer consideration here than they have met with in the course of the above general statement of results. These specially relate to those cases in which no development of commas occurred as the result of cultivations. Taking the entire series of primary plate cultivations of intestinal materials, it will be seen that they amounted to 22, that a development of commas occurred in 16, and that in six of them no commas were detected. It has been already pointed out that in one case the material cultivated was not of choleraic origin, that in two cases the nature of the material was doubtful, and that in two the nature of the substratum might be regarded as accounting for the phenomenon, so that only a single case remains in which an apparently unequivocal choleraic material cultivated in a seemingly favourable substratum failed to produce a recognisable crop of commas.

With regard to this latter case, however, I must here draw attention to three points. In the first place it must be allowed that the failure to detect commas in the cultivation does not absolutely prove their actual absence from it. This is due to the well-known fact that colonies of commas and other schizomycete organisms cultivated on agar-agar jelly fail to present the same constant, well-defined, specific macroscopic characters which they possess in common gelatine cultivations; so that, although it is in most cases possible to determine readily that certain colonies are not composed of commas, it is at the same time impossible macroscopically to distinguish those that are composed of commas from colonies belonging to certain other forms. This being so, it follows that where commatous colonies are only present in small numbers in a mixed cultivation, they may fail to be detected unless specimens from every individual colony present be examined. Now in the present instance this course was not followed; but, on the contrary, only a very limited number of colonies were examined due to the cultivation having been carried out at a time when a press of other work was in hand. In the third place, believing as I do in the polymorphic nature of the commas associated with cholera, I do not feel convinced that in any case such as this, where only a single primary cultivation was conducted, the mere absence of recognisable commas satisfactorily demonstrates the absence of the organism. Unless a succession of cultivations be carried out under conditions favourable to the appearance of commas, it is impossible to determine that any material, however free of actual commas, does not contain potential ones—does not contain forms which under certain conditions would be capable of becoming converted into or of giving origin to commas. In the present case colonies of minute straight bacilli abounded in the cultivation, and I do not think it impossible that these were developmentally related to commas.

The facts in regard to the case in which it was distinctly ascertained that the materials cultivated were really not of choleraic origin are the following. An extensive series of cultivations of normal alvine materials had led to the conclusion that the curved schizomycete organisms occurring in them, although often present in considerable numbers, were not reproduced in artificial cultivations in neutral or alkaline agar-agar jelly, and that they therein differed from those occurring in choleraic media. In order to obtain a sharply defined demonstration of the existence of this difference advantage was taken of the arrival of a supply of material from the hospital derived from a case, admitted as one of cholera, to start simultaneous plate cultivations of it and of a healthy alvine evacuation containing appreciable numbers of commas, on alkaline agar-agar jelly. On the following day both cultivations were carefully examined with the confident expectation of finding colonies of commas present on the one plate and absent from the other. In place of this, in spite of the most careful and extended examinations of colonies, no commas could be detected in either cultivation. There could be no doubt as to the presence of curved schizomy-

cete organisms in the original, supposed choleraic material, but there was certainly a failure in the development of any in the cultivation derived from it. At this stage another supply of material from the same case came over from the hospital. As a certain amount of suspicion had been aroused in regard to the nature of the case, the reaction of this material was tested and found to be acid. As, however, there was nothing else specially to distinguish it from other materials, which had yielded commas on cultivation, a fresh plate was set. On the following morning this was very carefully examined, specimens being taken from every form of colony which appeared in any way to present distinctive features, but in none could any commas by detected. The bed-head ticket containing the history and treatment of the patient was now sent for, and from it it was ascertained that, although admitted and treated for some time as a choleraic one, the case had subsequently been recognised as one of malarial fever. The phenomena in this case naturally led to a careful revision of all the others in which plate cultivations had failed to produce recognisable crops of commas, and, as has been already shown, in all save one it appeared that there were either reasons for suspecting the truly choleraic character of the material, or that conditions were present in the substratum to which the phenomenon might reasonably be ascribed.

One other case deserves some special notice, namely, one in which, whilst the original material appeared to be devoid of recognisable commas, a plate cultivation of it yielded a crop of them. The apparent absence of commas from the original material may have been due to their being present in very small numbers and to their having failed to stain well due to the nature of the material in which they were situated. That the latter condition may exert considerable influence in certain instances appears not to be improbable, for I have more than once observed that in cases like the one at present under consideration, where the flocculi were of a very gelatinoid mucous character, a special difficulty in obtaining satisfactorily stained preparations was encountered, the material seeming to obstruct the stains in penetrating the substance of bodies embedded in it. On the other hand, however, I believe that the failure to detect commas in the original material may have been really due to the fact that no such bodies were present in it, for it by no means necessarily follows that because a material contains bodies capable of giving rise to commas under favourable circumstances, it should contain actual commas as well. This point, which has already been alluded to, will be more fully considered subsequently.

Taking the entire series of observations on choleraic materials, the results certainly appear to indicate that the belief in the presence of easily cultivable commas as a very constant and characteristic feature in the dejections and intestinal contents in cases of the disease is a well-founded one. They also indicate the absolute necessity of cultivations in suitable substrata in order to the attainment of any definite conclusions in regard to the presence of bodies of such nature in any material, for they show in the first place that materials may

be either apparently or really devoid of actual commas and may at the same time be capable of serving as the source of cultivations containing them, and on the other hand that they may contain actual commas and yet fail to reproduce such bodies on cultivation under circumstances favourable to the development of those associated with choleraic media.

II.—On the presence of Curved Schizomycete organisms in non-choleraic materials.

A.—On the occurrence of curved schizomycete organisms in normal alvine evacuations.

An extended series of observations was carried out in regard to the question of the presence and characters of commas in such materials.

These included—1, Examinations of fresh materials ; 2, Cultivations.

1. Results of examinations of fresh, normal alvine materials.

Thirty-three distinct samples were subjected to examination, the specimens being prepared in the same fashion as the choleraic ones. In all of these without exception curved schizomycete organisms were present in varying numbers. The principal difficulty in regard to these materials lies in the enormous multitudes of multifarious schizomycete organisms present in them, which seem in truth to constitute the greater part of their mass and tend to crowd the preparations in bewildering profusion.

It therefore becomes necessary to dilute them in order to obtain satisfactory preparations or materials suited for cultivation. This dilution in the present instance was effected by means of freshly sterilised salt solution of 0·8 per cent. strength.

The number of commas present varied very considerably in individual instances, and in any case was very inconsiderable in relation to that of other schizomycete forms. They also showed very considerable variation in form. Many of them were long, slender, only slightly curved and with acutely pointed extremities. These resembled very closely the commas present in the case previously described as having ultimately proved to be of non-choleraic nature. Others, however, presented characters seemingly identical with those of the commas in typical choleraic media.

In some cases, minute vibrionic or spirillar forms were present in very considerable numbers, and in others large active and energetic specimens of Amœba coli and Trichomonads abounded.

2.—Results of cultivations of normal alvine materials containing commas.

In 18 cases primary plate cultivations were instituted. The substratum in the most of these was neutral or faintly alkaline, but in a certain number of

varying degrees of acidity. Colonies of various kinds were developed in abundance and in many cases presenting macroscopic features identical with those of commatous origin. Specimens were prepared from all colonies which appeared in any way to present distinctive features. The numbers of preparations from individual plates in consequence amounted in some cases to as many as ten, but in spite of this in not a single instance were commas of any kind to be detected. The forms most commonly developed in the cultivations consisted of short straight rods and micrococci, but in certain instances an abundant development of elongated bacilli occurred, the colonies of which were of superficial habit with peculiar lichenous, foliated margins. The variety of forms developed was curiously limited in relation to the multifarious nature of those present in the original materials. Cultivations were not, however, limited to primary plates, for having become more and more convinced of the polymorphic nature of the choleraic commas, and of the apparent relation which the appearance of special forms bears to variations in the conditions in which cultivations are carried out—the habit of growth acquired in one medium appearing to persist to a certain extent for some time in others of different constitution—repeated cultivations of materials derived from the primary plates were made on other plates and in tubes. The number of successive cultivations made in this way in individual instances was very considerable, in one case as many as seventeen successive ones being carried out, but, in spite of continued subjection to conditions favourable to the development of the commas associated with choleraic media, in no instance did such development occur here. The nature of the substratum and the course of cultivation were varied in different ways, the media being in some cases solid, in others liquid, the reaction being varied, the primary cultivations being sometimes carried out in liquids, the original materials for inoculation being sometimes employed whilst fresh, at others only after having been kept for varying periods, but in all cases the result was alike, there was no development of commas.

These results would appear to indicate either that bodies of the nature of the commas present in choleraic materials are entirely absent from normal alvine evacuations whether in actual or potential condition, or that, if they be present, that they are incapable of further development under conditions favourable to those of choleraic origin. They also point out the absolute necessity of cultivation experiments, showing, as they do, that the mere presence of curved schizomycetes can neither determine their true nature, nor the character of the medium in which they exist.

B. –On the occurrence of curved schizomycetes in the intestinal canal of Guinea-pigs.

Only one or two observations regarding this point were carried out. As has been pointed out by Dr. Klein, curved schizomycete organisms form a character-

istic feature in the contents of the cæcum of healthy guinea-pigs. They appear to be normally present in very considerable numbers. Only one series of cultivations from materials of this nature was attempted and in it no development of commas resulted.

C.—On the occurrence of curved schizomycete organisms in water.

The occurrence of commas in water has been frequently pointed out by other observers, and what I have to do here is merely to indicate the connection existing between certain conditions in the water of tanks in and around Calcutta with the presence of very large numbers of commas which resemble those associated with choleraic materials in size and form, and so far at all events in nature in being cultivable in agar-agar jelly.

At almost any season many of the tanks in and around Calcutta are more or less covered by a scum of Euglenæ, which is of a bright brick-red color in the morning, of a vivid green in the evening, and which is much less conspicuous and defined during the day than it is from sunset to sunrise. These variations in its characters are dependent on recurrent periodic changes in the condition of the component Euglenæ. The definition and specially the dry dusty aspect of the scum in the evening and early morning are due to the fact that at these times the vast majority of the Euglenæ are aerial and not aquatic organisms, the cells containing the then encysted and passive protoplasts being raised in various degrees above the surface of the water, and in the majority of cases being entirely removed from contact with it and projecting freely into the air. The relative inconspicuousness of it during the day is, on the other hand, due to the fact that they are then submerged and swimming free in the water. The changes in color are dependent on alterations in the relative amounts of red oily coloring matter, and specially in alterations in its distribution within the bodies of the protoplasts. The scum is not, however, solely composed of living Euglenæ, but, on the contrary, contains masses of the empty cysts and stems with dilated bases belonging to previous cycles of the encysted condition of the organisms. Bright, dry weather tends to induce constantly increasing thickness in this scum due to the fact that, under such circumstances, the normal cycle of developmental changes of form goes on recurring with unbroken regularity, and that accordingly, quite apart from any coincident processes of multiplication connected with the encysted condition of the organisms, there is necessarily a constantly recurrent addition of increments of dead matter in the form of empty cysts and stems. Heavy downfalls of rain, on the other hand, tend to cause it to disappear, due to the fact that they both break up the sheets of empty cysts and stems, and by driving the Euglenæ down into the water tend to prevent their normal assumption of an aerial habit. So long, however, as conditions remain favourable to the regular periodic succession of the diurnal and nocturnal phases in the life history of the Euglenæ a steady increase in the scum

goes on. Any scum of this nature, composed in considerable proportion of dead organic materials affords a favourable site for the development of both saprophytic and parasitic organisms, and we accordingly find it crowded with infusorial, monadinic, and schizomycete forms. Among the latter curved forms are frequently, and, under certain conditions, apparently normally present in very large numbers.

In order to obtain these commas with certainty and in large quantity, it is only necessary to procure a considerable mass of the scum, which can always be done readily at almost any time from the middle of the cold weather until the onset of the rains, and to introduce it into a jar of water. The conditions are here unfavourable to the continued life of the Euglenæ, and they consequently gradually die off, while a very thick scum consisting of their bodies in various stages of decomposition mingled with masses of their empty cysts and stems accumulates on the surface, so as sometimes to attain a thickness of about a quarter of an inch.

If portions of this be removed and their under-surfaces examined, they will be found to be more or less coated by a stratum of clear gelatinous material which is crowded with curved schizomycetes of various forms, but many of which precisely resemble those associated with choleraic media in their morphological characters.

In two instances in which cultivations of this material were carried out in feebly alkaline agar-agar jelly, a development of distinct commas occurred. The subject was, however, unfortunately not pursued further at that time (April) due to press of other work, and when again recurred to during the rains had to be abandoned due to the impossibility of obtaining good supplies of the material at that time. With the return of the cold weather it will, however, be again possible to take it up and as common gelatine media will then be available for cultivations, the physiological characters of the commas may be more accurately compared with those of the choleraic ones than would have been possible at times when agar-agar jelly has to be substituted for them. In the meantime the results, so far as they go, must be regarded as enforcing the necessity of caution in coming to hasty conclusions in regard to the interpretation to be put on the phenomenon of the presence of cultivable commas in sources of water supply.

III.—On variations in the form and course of development of the Curved Schizomycetes associated with choleraic materials in connection with variations in the conditions to which they are exposed.

In taking up this part of my subject I am well aware that the conclusions at which I have arrived, whilst in accordance with the views of many botanical workers, are in direct conflict with those of the majority of pathological bacteriologists, and that I am therefore laying myself open to much adverse criticism in publishing

them. This, however, is no sufficient ground for omitting to notice the results which a series of careful observations have appeared to lead to.

Attention was primarily directed to this subject first by the observation that certain modifications in form appeared to characterise many of the commas which had been developed in substrata which had not been fully neutralised, and which therefore retained a slightly acid reaction, and secondly by the occurrence in a series of alkaline tube cultivations of a gradual development of a series of forms similar to those described and figured by Dr. Klein as characteristic of agar-agar cultivations conducted at the ordinary room-temperature in winter in London.

In the case of several plate cultivations on faintly acid agar-agar jelly it was noticed that, whilst commas were abundantly developed, many of them when compared with the typical slender commas of neutral or alkaline cultivations were characterised by their relative thickness and shortness. On examining them more closely, too, such commas were in many instances observed to present appearances which were for some time regarded as indicative of the incipient stages of spore formation. These consisted in the presence of colorless areas in the substance of the stained protoplasm (Plate III, Fig. 1A). The number of such spaces varied in individual instances, being in great part apparently related to the size of the commas in which they originated, short specimens containing only one colorless area whilst longer ones contained two or even three. In general appearance they very closely resembled spores in an early stage of development, but, in spite of the most careful observation, no further evidences of spore-formation ever occurred, and it ultimately became evident that they were not due to any process of that nature but to one of mere vacuolation of the protoplasm.

Numerous observations were now undertaken with a view to determine more accurately how far variations in the character of the substrata appeared to be capable of influencing the form of commas developed in them. In the course of these observations two distinct series of experiments were tried. In the first of these, cultivations — both on plates and in tubes — were simultaneously started from a common source, consisting of a pure tube cultivation of commas, in media of various reactions and the characters of the resulting growths carefully compared with one another. In the second series the effect of repeated alterations of various reactions was studied, materials derived from alkaline or neutral substrata being cultivated in acid ones, and the resultant crops being used as a source of materials for fresh alkaline cultivations.

The result of the first series of experiments was to show that while in neutral or feebly alkaline media crops of typical slender commas were developed, in the case of acid cultivations these were more or less replaced by shorter and relatively thicker forms, which in many instances ceased to shew any morphological evidence of their commatous origin. On comparing the results of parallel cultivations in feebly and strongly acid media corresponding differences in degree of deviation from the standard alkaline type regularly manifested themselves.

In feebly acid cultivations a certain, and sometimes a considerable, proportion of typical commas was present associated with others of shorter, relatively thicker form and in many instances containing vacuoles. In the larger specimens of these abnormal commas, the characteristic curvature was still distinctly recognisable, but these were connected by a series of insensibly gradated forms with bodies which no longer showed any traces of curvature and which presented the character of rounded or oval cocci with or without a distinct central vacuole. Such bodies occurred either isolated or in pairs, or in certain instances in connected series of various numbers so as to give rise to rod-like bodies. The second series of experiments yielded corresponding results. The transfer of materials derived from pure cultivations of commas in alkaline or neutral media to acid substrata resulted in the development of bodies modified as described above in varying degree according to the character of the substrata as feebly or strongly acid. Successive cultivations in strongly acid media resulted in the development of crops almost entirely, or entirely consisting of the modified coccal forms, and successive cultivations of materials of this nature in alkaline media led to a gradual replacement of the coccal forms by commas and the ultimate appearance of pure cultivations of typical commas. These results were repeated with methodical and extreme regularity as to leave no reasonable ground to doubt that they really indicated a considerable range of polymorphism as a character of the choleraic commas, but the precise developmental relations of the various forms to one another remained for some time a matter of obscurity.

At length, however, a set of cultivations presented itself which afforded means for clearing up this question. This, as has already been mentioned, was one in which the substratum was alkaline and in which peculiar abnormal forms, similar to those described by Dr. Klein, formed a very conspicuous feature. Dr. Klein's observations had clearly shown that the development of vacuolate forms was a phenomenon appearing under the influence of certain conditions in alkaline cultivations. According to his experience the condition which appeared most closely related to it was depression of temperature. In those cases in which it was observed in Calcutta it did not appear to be so much primarily related to thermal conditions, for it occurred in some cases in cultivations conducted during the height of the hot season, as to prolonged cultivation in one medium. As a rule vacuolate forms were either absent or only present in very limited numbers in specimens derived from recent neutral or alkaline cultivations, and where they appeared conspicuously did so in cultivations of some duration and in constantly increasing numbers as time advanced.

This was very distinctly the case in one cultivation. What rendered this cultivation particularly instructive was, however, not so much the numbers in which the vacuolated forms occurred as the exceptionally large size to which many of them attained, which rendered it possible with comparative ease to follow out the various modifications of form and to recognise the developmental relations which the various forms bore to one another. Both the distinctly vacuolate

forms and the typical commas present in the cultivation showed very great range of variation as to size, some being very minute, some of relatively colossal proportions, and an insensibly gradated series of intermediate forms serving to connect those presenting extremes of magnitude with one another. The only difference appreciable between the largest and smallest specimens of the various forms was one of size. The vacuolate bodies varied very greatly in form, in some cases being circular, in others almost semi-circular, in others elliptical or fusiform, and in still others curved. Some of the curved forms clearly presented the characters of commas including one, two, or three vacuoles. Others were thickened in varying degree by filling up of the curve, and where this had advanced far they necessarily more or less presented semi-circular, hemi-elliptical, or semi-fusiform contours according to the character of the curve of their convex contour. All varieties of vacuolate forms were accompanied by corresponding non-vacuolate bodies in varying numbers. The circular, fusiform and elliptical specimens occurred either singly or associated in pairs. The degree of vacuolation differed greatly in individual specimens, the vacuolar area in some cases being relatively very large, in others extremely small. In the former case the precise distribution of the colored protoplasm varied in different instances. In the case of the circular, and fusiform bodies the vacuole was centrally situated and the protoplasm formed a more or less even marginal band. In the elliptical ones the arrangement was sometimes of the same nature, at others the protoplasm appeared to be accumulated at the opposite poles, being resolved into two seemingly distinct portions separated from one another by the vacuolar space. In the case of the commatous, and the semi-circular or other plano-convex forms the vacuole was either centrally situated or in immediate relation to the concave or plane edge. In those commas with a central vacuole the protoplasm was in some cases resolved into two distinct polar bodies, while in others it formed a continuous rim (Plate III, Fig. 1B).

All the vacuolate forms above described were accompanied by a corresponding series of other forms showing various stages in the process of their resolution into commas of various sizes. In the case of the circular bodies of this class in most instances the peripheral rim of protoplasm could be seen in different stages of resolution into a pair of commas, due to the occurrence of a solution of continuity at two distinct points. The resulting commas were in some cases of approximately equal sizes, in others one was larger than the other due to the solution of continuity not having taken place at corresponding points in the protoplasmic ring. Specimens were present showing varying degrees in the process of dissociation of the pairs of commas. In many cases they remained for some time adherent to one another by one extremity and where they had done so and at the same time extreme divergence of the opposite free extremities had occurred figure of three or even V shaped bodies were present. In other cases, especially in those where the process of resolution tended towards the development of commas of unequal size, convergence in place of divergence

of the free extremities to a greater or less degree had led to the formation of bodies of concentrically spiral type (Plate III, Fig. 1).

Corresponding series of forms showing the process of resolution in ellipses and spindles were also present in abundance. As in the case of the circles the commas resulting from the process were either of equal or unequal sizes. Where the latter was the case the smaller comma often appeared as though included more or less within the concavity of the larger one (Plate III, Fig. 1).

Very remarkable and instructive appearances were presented in many instances where the process of resolution had affected one of the compound bodies consisting of two spindles united in pairs by their opposed extremities. Where the process of resolution had advanced in like degree in both spindles, and where divergence of the distal extremities of the pairs of resultant commas had occurred, the entire body presented the appearances represented in the accompanying figure (Plate III, Fig. 1B).

Where, on the other hand, resolution had advanced more rapidly in one or other spindle, and where, therefore, divergence had only begun to occur in one of the resultant pairs of commas, this figure was replaced by that also shown in the figure (Plate III, Fig. 1B).

Specimens were also present which appeared to show that in those cases in which the vacuolation had led to a resolution of the protoplasm into two distinct polar masses, each of these tended to become gradually converted into a short comma due to gradual hollowing out of its equatorial face. In this form of resolution the long axes of the resultant commas will of course be directed at right angles to the long axis of the parent body in place of parallel to it as in other varieties of the process. Where vacuolation was lateral in place of central only one comma appeared to arise as the result of the occurrence of resolution, arising in consequence of gradual conversion of the continuous lateral band of protoplasm and solution of the vacuolate side of the parent body, the process being essentially one of rejuvescence and not of multiplicative subdivision of protoplasm.

The phenomena presented by the various curved forms were parallel to those occurring in connection with the elliptical and fusiform ones. In some cases two commas were developed, in others only a single one corresponding with the convex surface of the parent. Where two commas resulted from the resolution of a parent body of like form, so long as they retained a definite relation to one another they formed a pair differing from those due to division of spindles, ellipses, or circles in having the convex and concave faces of both the commas directed to the same side in place of being opposed to one another (Plate III, Fig. 1). Where processes of resolution affected plano-convex forms they appeared usually to lead to the formation of a long strongly curved body and a short straight one corresponding respectively to the convex and plane faces of the parent. In many cases, however, division appeared to be replaced by

rejuvescence and the development of a single, large comma only, corresponding with the convex surface of the parent.

All these points could be very readily made out in connection with the larger specimens of the various forms, and when once this had been done the fact that similar processes took place in the case of the smaller ones also, appeared evident on careful examination under sufficiently high magnifying powers.

The material containing all these various forms was next employed to initiate a series of tube cultivations, in substrata of various reactions with the following results :—

Cultivation I.—Substratum alkaline.—The resultant crop consisted primarily in greater part of typical slender commas, many of them being of very minute size. A certain number of small round and elliptical cocci were also present and in many of these vacuolation and appearances corresponding with various stages in the process of resolution into pairs of commas were clearly evident.

Cultivation II.—From cultivation I; substratum strongly acid.—The growth in this case was mainly composed of cocci. These were either isolated, or associated in pairs, or in series so as to form compound bodies of rod-like form. Very few typical commas were present, but many of the longer cocci showed more or less evident concavo-convex contours. Many of the cocci and some of the distinct commas were vacuolate.

Cultivation III.—From cultivation II; substratum strongly acid—The growth here resembled in its characters that of the previous cultivation, but the proportion of distinct commas was even smaller. Vacuolation was prevalent.

Cultivation IV.—From cultivation III ; substratum alkaline.—In this cocci of various forms abounded, many of them shewing various stages of vacuolation and resolution. Very minute commas in many cases associated in pairs were also present in large numbers.

Cultivation V.—From cultivation IV; substratum alkaline. The growth in its general characters resembled that of the previous cultivation. Some commas of fair size were, however, present among the minute ones.

Cultivation VI.—From cultivation V; substratum alkaline.—The results here were very instructive, commas of various sizes were present in enormous numbers mingled with a certain number of coccal forms in various stages of resolution into pairs of commas.

Cultivation VII.—From cultivation VI; substratum strongly acid.—The characters of the growth here were similar to those in cultivation II.

Cultivation VIII.—Also from cultivation VI; alkaline and so much diluted as to remain permanently fluid.—The growth in this consisted of a splendid crop of long vibrionic filaments composed of commas adhering to one another by their extremities mingled with a certain number of isolated commas. Various other series of cultivations gave results similar to those detailed above, and careful and repeated examinations of preparations derived from them have only

served to confirm the belief in polymorphism as a character of the choleraic commas under the influence of varying conditions. There can be no question regarding the correctness of Dr. Klein's observation relative to the occurrence of processes of multiplication by division of a type differing from the ordinarily recognised transverse one. In some cases the processes are of distinctly longitudinal type and the long axes of the resulting commas then follow the same direction as that of the parent body, where this is of elongated form. I am strongly inclined to believe that processes of this character are not peculiar to the choleraic commas, but will be found to occur in the case of other schizomycete organisms. In a preparation of large oval cocci, developed in a cultivation of materials from a diphtheritic patch on the surface of a wound in a case of erysipelas, which is now in my possession, there are, I believe, clear evidences of the occurrence of similar processes of longitudinal division.

In other cases the process of division is primarily transverse, but at the same time differs from the commonly recognised process in details and results. In them we have transverse division of the protoplasm as the result of extensive vacuolation, and the development of the resultant commas is such that their long axes are transverse to that of the parent.

These processes of multiplication of commas are initiated by processes of vacuolation leading to gradual sub-division of the originally continuous protoplasm, and such vacuolation only occurs under certain circumstances. It tends to appear, according to Dr. Klein's observations, in association with depression of temperature; and, so far as I have been able to ascertain, it is also associated with partial exhaustion or acidity of the substratum. In recently started alkaline or neutral cultivations conducted at a temperature of a certain degree of elevation, such processes of vacuolation and division do not occur, and continuous processes of transverse division of the ordinarily recognised type lead to the accumulation of typical commas. Where processes of vacuolative division have been established, they may be caused to disappear by cultivation under the conditions favouring the ordinary processes of multiplication. This result, however, does not necessarily occur completely at once. A varying number of successive cultivations may have to be carried out ere these abnormal forms and processes are completely replaced by the typical ones. It would appear that we have here indications of the establishment of a special habit of growth in connection with certain conditions and tending to be propagated hereditarily for some time under other circumstances.

On the other hand if successive generations of the organism are continuously exposed to the influence of the conditions favouring the abnormal processes an opposite result ensues. There is a progressive tendency to disappearance of the typical forms and to the substitution of abnormal ones differing more and more from the original type. Taking a material consisting of typical commas as the primary source of a series of successive cultivations, we obtain various results

according to the nature of the substrata employed. If the initial cultivation be conducted in an acid medium, we obtain a growth in which typical commas are to a great extent replaced by vacuolate commas and cocci of various forms. If material from such a cultivation be cultivated in alkaline or neutral media the abnormal forms tend to disappear due to the development of typical commas due to the ordinary processes of transverse division and to resolution and division of the vacuolate forms. On the other hand if the substrata be acid, the commatous forms come more and more to be replaced by vacuolate and coccal ones. This is due to the fact that under such conditions, whilst processes of vacuolation and of division of protoplasm continue to occur, the secondary protoplasts have less and less tendency to assume the characters of commas and more to repeat those of the parent cocci.

Vacuolate cocci or short commas, in which the process has advanced so far as to lead to division of the protoplasm into two distinct polar bodies, will, if placed under certain circumstances, give origin to pairs of commas, and if placed under other conditions to pairs of cocci. Where the conditions are such as to favour growth of a coccal type, the resulting cocci may either separate from one another or may remain united in pairs or linear series. When they remain united a series of forms arises which constitute a parallel to the vibrionic forms which appear under certain conditions in growths of the commatous type.

Such are the conclusions at which I have arrived from observations and experiments of the nature described above. Should they prove to be well grounded, they will only afford another proof of the absolute necessity of cultivations carried on under distinctly defined conditions in order to any satisfactory determination of the question of the presence or absence of schizomycetes of particular nature in any suspected material.

A few special points still remain to be considered. The first of these relates to the macroscopic features presented by cultivations of the choleraic commas in agar-agar jelly. These are not at all of the specific and distinctive type described as characteristic of cultivations carried out in common gelatine media. There is in no case, so far as I have observed, any tendency to liquefaction of the basis in the neighbourhood of the colonies. In plate cultivations the latter are usually evenly rounded, sharply defined and of considerable size; sometimes they present a slightly lichenoid appearance. In many cases they attain a diameter of several millimeters. In one case such defined, colonies were replaced by a general diffused haze pervading large areas of the plate. In many cases the colonies are absolutely indistinguishable macroscopically from those belonging to other forms of schizomycetes with which they are associated. Like many other colonies composed of rod-like units they present a peculiar bluish tint when the plate is held obliquely so as to subject them to a certain amount of transmitted light, whilst the majority of colonies of coccal origin under similar circumstances usually show a pinkish, yellowish, or whitish color.

The macroscopic characters of tube cultivations also are neither specific nor constant. In some cases the track of the needle is sharply defined and opaque and surrounded either by a series of translucent prominences, which in profile view form a wavy frill, or by a continuous translucent cloud with well-defined margins, and acutely conical or clavate inferiorly. In other cases the needle track presents a number of separate, very minute opaque points and is acutely conical, wide above and tapering off inferiorly to a point. A translucent cloud, like that just described, is sometimes present in these cases also. In acid cultivations it was usually absent and the needle-track being of the tapering character just described, the entire growth, including the surface halo around the orifice, presented a tack-like figure. Some of the varieties of the growth, especially that with defined track and frilled edges, were absolutely indistinguishable from those of certain other kinds of schizomycete organisms.

The entire series of cultivations demonstrated the correctness of the statement that, so far as macroscopic characters go, agar-agar jelly is not a favourable medium for the manifestation of specific physiological properties of the schizomycete organisms cultivated in it.

The influence of the substratum came out very clearly in connection with the appearance of filamentous forms consisting of commas united in linear series. Such forms were only very rarely developed in solid media and then only in very small numbers. In fluid media, on the other hand, they formed a conspicuous feature, more especially in dilute agar-agar solutions, in which in some cases to a great extent, they replaced the common isolated commas. In no instance did the filaments appear to be of truly spiral type. They were merely sinuous or undulated in various degrees according to the amount of curvature in the component commas.

The individual commas were, as a rule, of considerable size. The outlines of the filaments in some cases were alike on both sides due to the concave surfaces of the component commas being directed alternately to either side (Plate III, Fig. 1C.) In other cases there was an absence of bilateral symmetry and the convexities and concavities of the commas were throughout directed to one or other side. Here, of course, one side of the filament presented a series of rounded, and the other of acute prominences (Plate III, Fig. 1C.) These characters were, however, by no means necessarily constant throughout the entire length of a filament, the arrangement of the commas on which they depended frequently varying from place to place. It does not appear quite clearly which arrangement ought to be regarded as the typical one. Where the multiplication of commas is of the common transverse character, it is natural to suppose that any filamentous form origination as the result of continued adhesion of the commas to one another will be of unilateral type. The same will naturally be the case in the case of the short filaments representing the primary pair of commas which have arisen as the result of processes of longitudinal division in parent bodies of bi-convex outline. Where, on the

other hand short filaments arise as the result of similar processes of longitudinal division and divergence in concavo-convex bodies, they will necessarily be of bilateral type.

But filaments of either type may also very possibly arise secondarily as the result of processes of torsion at the points of union of adjacent commas, so that in any case it must remain uncertain how far the characters presented by any given filament ought to be regarded as of primary developmental value, or as mere indices to previous exposure to certain external influences.

Microscopic examinations of Intestinal, Splenic, Hepatic, and Renal tissues were only carried out in one or two instances. The number was much too limited to permit of any definite conclusions being arrived at, but in no case did there appear to be any evidence of the presence of special schizomycete organisms in the substance of the tissues.

CALCUTTA,
25th September 1885.

On the presence of peculiar parasitic organisms in the tissue of a specimen of Delhi Boil.

BY

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In the heading given above it will be observed that the old term "Delhi Boil" has been employed in place of the mere comprehensive one "Oriental Sore." The local designation has been deliberately reverted to, because it appears to be quite possible that growths presenting the generic characters of Oriental sore may be due to distinct specific causes in different localities. The neoplasm in the present instance was certainly associated with, and very possibly due to, the presence of a peculiar form of parasitic organism. Apart from this, however, it presented no specific characters, but was essentially a simple granuloma such as might arise in connection with the presence of persistent irritation dependent on very various causes. In employing the term "boil" rather than "sore" I have been guided by the desire to indicate clearly from the outset that the parasitic bodies in this case occurred independent of the existence of any raw surface—of any solution in the continuity of the cutaneous surface—and were present in the initial stage of the disease, in which there is merely an accumulation of granulomatous neoplasm within the dermal tissue.

There can be no occasion here for considering at length the general conditions in association with which affections of this character arise in India, as this was fully done a few years ago by Dr. Lewis and myself in a paper on "The Oriental Sore," since the publication of which no important additions to our knowledge of the subject have been made. I shall accordingly confine myself almost entirely to giving an account of the structural features of the specimen under consideration. This was obtained from Delhi, where it was excised from the person of a patient in the dispensary, placed at once in absolute alcohol and sent down to me in Calcutta for examination. In the hard and shrunken condition in which it arrived it measured one inch in length by 0·416 of an inch in maximum thickness. It presented the usual bluntly convex contour, and, as

already mentioned, had not yet commenced to ulcerate, so that the term boil rather than sore was strictly applicable to it.

Vertical sections, including the entire area of the specimen and stained with picrocarmine, presented the following features when examined under low powers (*vide* Fig. 1). In the first place it was evident that the portion of tissue which had been excised did not include quite the whole of the diseased area, for although at one side the skin presented fairly normal characters, at the other a continuous invasion of its substance by granulation-tissue was very evident. The most conspicuous features were the accumulation of great masses of granulation-tissue, especially in the upper part of the dermis, and excessive hypertrophy of the superjacent epidermis. These appearances attained their maximum development in the most prominent portion of the growth and gradually tailed off on either side. On that side where the limits of the diseased area appeared to have been approximately reached, the continuous stratum of granulation-tissue present in the central portions became replaced by isolated patches of various size separated from one another by normal dermal tissue, and the hypertrophy of the epidermis gradually diminished and disappeared. On the other side a tendency towards the same thing was evident, but, even at the very extremity of the section, a thin stratum of continuous granulation-tissue and a certain degree of epidermal hypertrophy could be readily recognised.

Both horny and mucoid strata of the epidermis showed clear evidences of varying degrees of hypertrophy. The former in the most prominent portion of the growth was about 0·01 of an inch in thickness, whilst at the relatively normal extremity it was only about 0·001. The mucoid stratum was enormously hypertrophied, especially as regarded its interpapillary portions. Towards the lateral portions of the sections there was very considerable thickening of it over the summits of the papillæ. But centrally this tended to diminish greatly and in certain sites an absolute atrophy seemed more or less to replace it, so that the papillary tissue came to be almost or actually in contact with the under-surface of the thickened horny layer, and it appeared evident that by a very slight extension of the process a raw surface would have necessarily been established. The thickness of the suprapapillary stratum at the extreme margin of the specimen, where it presented the most normal characters, amounted to about 0·001 of an inch, while in the sites of its greatest development it was about 0·006. The interpapillary processes varied extremely in size and form. At the relatively normal margin of the growth, the thickness from the extremity of the processes to the surface of the horny stratum was about 0·009 of an inch; in the most prominent portions it in some cases amounted to about 0·07 of an inch. In some cases the prominences were broad, in others narrow; in some pointed, in others distinctly bulbous; in some they were simple, in others irregularly branched inferiorly.

Immediately beneath the epidermal stratum there was a great mass of

granulation-tissue which projected upwards into the papillæ and formed a continuous layer beneath them. Towards the lateral portions of the specimen this tissue gradually diminished in amount, disappearing from the papillæ, and occurring beneath them in isolated patches only. In the more advanced central portion of the tumour, the neoplasm appeared entirely to replace the normal dermal tissue in the area which it had invaded.

This continuous stratum of neoplasm beneath the papillary layer in its thickest portions was about 0·06 of an inch deep, and from this it gradually thinned out laterally in both directions. Its inferior margin was in some places fairly even; in others, on the contrary, it presented a series of irregular processes projecting for varying distances into the subjacent dermal tissue and there becoming continuous with a system of irregular masses of similar composition scattered throughout the interspaces of the fibrous bundles. In some cases these processes could be readily recognised as specially related to hair-follicles or to the ducts of sweat-glands.

The complete continuity of the subpapillary granulation-tissue in its more developed portions was only interrupted by the bundles of muscular tissue belonging to hair-follicles which had completely disappeared. In less advanced portions hair-follicles in various stages of degeneration and disintegration were also recognisable.

Immediately beneath this continuous stratum of neoplasm was one of modified dermal connective tissue in course of invasion by the growth. Due to the presence of abundant masses of unaltered connective tissue in it the sections of it stained with picrocarmine presented a conspicuous deep, bright red colour, contrasting strongly with the pinkish yellow of the granulation-tissue above it. Interposed between the bundles of connective tissue there were everywhere masses of granulation-tissue of various sizes and the most irregular outlines. In some cases these were specially related to hair-follicles, sweat-glands, or the adventitia of the larger vascular trunks, but in most they were quite irregularly distributed and appeared as it were to form a natural injection of the lymphatic spaces of the normal tissue. The appearances seemed to indicate that where hair-follicles have become involved in masses of the neoplasm they are comparatively rapidly disorganised and soon disappear. The same would appear to be the case with the straight portions of the sweat-tubes, but the convoluted extremities, on the contrary, in many instances seem to persist unaltered even where entirely buried in masses of the growth (Plate III., Fig. 3).

The extent to which the fibrous tissue of the dermis was broken up and, as it were, dissected by the interposition of these masses of the new growth varied greatly in different parts of the tumour. Towards the centre masses of the neoplasm were present in very great numbers and tending more and more to become confluent with one another. Well-stained sections very clearly demonstrated the various stages in the process of separation of the bundles from one

another and their subsequent resolution into their constituent fibres, the appearances being similar to those present towards either extremity of the continuous stratum. This process of interposition of masses of granulation-tissue and the consequent dissociation of the normal dermal elements necessarily gives rise to very considerable thickening, and it is to it along with the constantly increasing thickness in the accumulation of continuous granulation-tissue above, together with the associated epidermal hypertrophy, that the gradual increase in prominence of the affected area of the skin is due.

Beneath the layer of modified dermal tissue the sections included fat-lobules of the subcutaneous areolar tissue, which, like the structures above them, showed in varying degrees distinct evidences of invasion by granulation tissue. In the lobules immediately abutting on the dermis masses of the neoplasm in the latter spread continuously into the substance of the lobules, dissecting the fat-cells apart from one another, pressing upon them in varying degree, and ultimately obliterating and replacing them entirely throughout considerable areas. Scattered and, so far as the sections were concerned, isolated patches of various size were also scattered throughout the lobules in varying numbers. In many cases these seemed to be related specially to the adventitia of the larger lobular vessels; in others, however, they appeared to be scattered at random among the fat-cells. Even in the very deepest portions of the specimen such isolated patches of neoplasm could in many places be recognised in the lobules (Plate III., Fig. 2).

Examinations of similar sections under higher microscopic powers confirmed the information attainable by means of low ones and revealed additional points in regard to structural details. The essential feature in the diseased area was clearly shown to consist in an accumulation of lymphoid and epithelioid cells among the normal tissue elements. When Dr. Lewis and I previously studied the subject, we were inclined to regard the morbid process essential to the disease as one in which a development of tissue of an adenoid type occurred. Now, however this may be so in certain cases, it most certainly is not necessarily the case in all, for in the present one there was no evidence of the development of any new stroma—of any new fibrous network. The most careful examination of sections both before and after brushing showed nothing, save an accumulation of lymphoid and epithelioid elements between the normal connective tissue structures of the dermis and subcutaneous areolar tissue, together with the results of associated processes of epithelial hypertrophy. As has been already pointed out, the morbid process in the dermis consisted essentially in a simple opening out and dissociation of the normal tissue elements by masses of pure granulation-tissue. The ultimate result of any such process must naturally be, where it advances sufficiently far, to replace the dense connective tissue of the dermis by one consisting of a stroma of separated, but persistent fibres including dense masses of cellular elements. The size and form of the spaces in the stroma

naturally varies greatly in different instances; in some they are relatively very large, as they must almost inevitably be where corresponding to interfascicular areas; in others they are much smaller and include only small masses of cellular elements, which may be either spread out in simple strata so as to form plates, or be heaped upon one another in irregular masses. Plates consisting of a single stratum of cells will naturally on vertical section appear as linear series of cells, whilst the forms presented by the aggregates of other nature will necessarily vary with the relation which the plane of the section bears to their actual plan. A certain number of fusiform protoplasmic bodies resembling the nuclei of typical connective tissue corpuscles may no doubt be recognised throughout the substance of the neoplasm (Plate II., Fig. 5). The number of such bodies in the present case was, however, very trifling, and it appeared much more reasonable to regard them as mere persistent elements of the normal fibrous tissue than as indices to the occurrence of any processes of development of a neoplastic stroma. Repeated and careful examinations of very many sections treated with numerous distinct staining reagents have only served to convince me more and more strongly that, if any development of new fibrous elements occurred at all in connection with the diseased process in this case, it could only have been very trivial, and that the real stroma of the neoplasm consisted merely of dissociated elements proper to the normal tissue of the part. The characters of the specimen appear to me clearly to show that a development of adenoid tissue is not necessarily a feature in the disease; they do not, however, indicate that such a process may not occur in certain, or even in the majority of, cases.

The cellular elements forming the essential feature in the neoplasm vary considerably both in size and outline (Plate II., Figs. 9—14). This is apparently to a considerable degree determined by the varying degrees of pressure to which they have been exposed during and subsequent to their development. In those sites in which they have not accumulated to any great extent, where they lie more or less separately in the spaces in which they are situated, many of them are more or less rounded in outline, or where they present an angular contour, are of considerable size and epithelioid character. Where, however, a greater accumulation has occurred, and where, therefore, the cells within the meshes of the stroma have come to be closely packed together, they are almost without exception of small size and more or less angular from mutual pressure. Where the inter-spaces in the stroma are of small size and the accumulation of such minute angular elements is relatively excessive, the outlines of the individual members of the aggregate may be very hard to distinguish, and the entire mass may thus come closely to simulate a single, large, polynucleate cell (Plate II., Fig. 11). Such densely aggregated masses of cells, as was previously pointed out by Dr. Lewis and myself, appear to form one of the sources from which the peculiar concretionary bodies occurring in the discharges in cases which have passed on into ulceration are derived. Truly polynucleated elements appeared to be entirely absent, and even binucleate ones were very rare.

The average size of a series of cells which were carefully measured was $8{\cdot}8\mu \times 7{\cdot}2\mu$, but the variations between individual specimens was very considerable, the largest ones measuring as much as $10{\cdot}7\mu \times 8{\cdot}5\mu$, the smallest only $5{\cdot}4\mu \times 5{\cdot}4\mu$. The size of the nuclei was much more constant to an average of $5{\cdot}6\mu \times 3{\cdot}4\mu$. In any case they were relatively large, and in the case of the smaller elements the layer of cytoplasm was so thin as to cause them in many instances to simulate free nuclei.

Examinations under high powers also demonstrated the various stages in the development of the growth very clearly, showing the gradual accumulation of lymphoid elements with progressive dissociation of the normal structures of the invaded area. The appearances presented in many of the fat-lobules of the subcutaneous areolar tissue were particularly instructive (Plate III., Fig. 4). In some places only a few scattered lymphoid and epithelioid elements were present between the fat-cells; in others distinct local accumulations had occurred; in others such accumulations had led to separation and partial compression of the surrounding cells, and in still others continuous masses of lymphoid and epithelioid elements had entirely replaced the fat-cells throughout considerable areas of the affected lobules. The adventitia of the lobular vessels, as before mentioned, appeared in many instances to form the primary site of invasion, but in others this was not the case, the adventitia remaining quite free even where other portions of the lobule had been seriously affected.

In the areas of invasion of the dermal tissue proper appearances corresponding with a natural injection of irregularly ramified lymphatic spaces were in many cases most distinctly traceable. In other places the lines of invasion had clearly primarily followed the tracks of hair-follicles or the ducts of sweat-glands. The various stages leading to the formation of the epithelial concretions occurring in the discharges of ulcerating specimens of the disease in association with the other form of concretions previously alluded to, could be readily traced as connected with processes of continuous growth occurring in the tips of interpapillary processes, or of detached portions of hair-follicles which had become isolated and buried in masses of the granulation-tissue (Plate III., Figs. 5, 6).

It is unnecessary here, however, to go into further details regarding the general structural features of the specimen, as these fully agreed with those described by Dr. Lewis and myself in our previous paper, and I shall therefore now go on to the special consideration of the characters of the peculiar parasitic bodies present in it, and which it appears at all events possible were the primary cause of the disease.

The specimen was primarily procured with the view of ascertaining whether there were any evidence of the association of any special form of Bacterial organisms with the diseased tissues. A very large number of sections were accordingly prepared with the freezing microtome and were subsequently subjected to the influence of various staining reagents—Picrocarmine, Logwood,

Gentian violet, Methyl blue, Spiller's purple, Bismarck brown, Fuchsin, Eosin, and Gibbes' double-staining fluid.

The specimens thus prepared were subjected to careful and methodical examination, but in none of them could Schizomycete organisms of any kind be detected. In none of them, save those which had been stained with Gentian violet, did any structural features manifest themselves beyond those which have been just described above. In those, however, which had been first thoroughly stained with Gentian violet and then washed out in spirit until almost all colour had disappeared from them, large numbers of peculiar violet or blue bodies appeared conspicuously among the surrounding, almost colourless masses of lymphoid elements (Pl. I., Figs. 1, 2; Pl. II., Fig. 8). Repeated and careful examination of a large series of sections prepared in a similar fashion revealed the constant presence of such bodies and furnished the data for the following details regarding their structural features and distribution.

In preparations stained with any of the other dyes mentioned above, with the exception of Fuchsin, they were absolutely indistinguishable even after a complete familiarity with their appearance and distribution had been attained. In some Fuchsin preparations faint indications of their presence could be recognised, but it was only by means of Gentian violet that they were rendered conspicuous and their characters sharply defined. Double staining with Gentian violet and Fuchsin in some cases gave very good results as will be further alluded to presently.

They varied very considerably in size. The average diameters of a series of measured specimens were $12{\cdot}6\mu \times 8{\cdot}8\mu$, the largest measuring $12{\cdot}8\mu \times 25{\cdot}6\mu$, the smallest $6{\cdot}4\mu \times 6{\cdot}4\mu$. Such minute specimens as the latter were, however, rare, and, as a rule, they were considerably larger than the lymphoid elements among which they were situated. Their form also varied greatly,—in some cases they were circular, in others elliptical, in others irregularly lobate (Pl. I. Figs. 2, 3, Pl. II. Figs. 3, 5, 6). Their contour was in the majority of instances smooth, but in some of a more or less tuberculate character. In some specimens a very delicate cell wall was clearly visible; in others it was wholly unrecognisable, or only to be detected on careful and special scrutiny.

The distinctness with which they appear in sections treated with Gentian violet is due to the elective staining of the nucleoid bodies which they contain by the dye. The number of such bodies present in different cells varies extremely,—in some cases only a single great nucleoid mass is present, seemingly occupying almost the entire cell body, in others a few of very various sizes occur, and in still others a large number of minute and fairly equal-sized ones are thickly scattered throughout the entire cell (Plate I., Fig. 3; Plate II., Figs. 3—7). The cytoplasm in the Gentian violet specimens remains almost uncoloured; in those in which Fuchsin has likewise been employed, it frequently shows a more or less pronounced red hue. The tuberculate appearance presented by some of the

cells is due to the numbers and size of the nucleoid bodies present in them, which in association form a mulberry-like mass pressing upon the cell wall and moulding it to the inequalities of its surface (Plate II., Fig. 2). Such tuberculate bodies on superficial examination present certain points of resemblance to the characteristic bodies in cases of Actinomycosis. On closer examination, however, it is evident that they correspond structurally with the description given above and are not due to any radiate aggregation of filaments.

In certain cases appearances apparently corresponding with the occurrence of processes of cell division are present, the bodies of the cells being strongly constricted so as to form two lobes connected by a narrow neck, or two distinct cells occurring which, from their relations to one another and the character of their opposed surfaces, seem to have just arisen due to the completion of such a process (Plate II., Figs. 4,5). In many instances, too, a distribution of the cells in little groups separated from one another by comparatively wide areas of granulation tissue can be recognised, and may possibly be indicative of the antecedent occurrence of processes of division (Plate I., Fig. 1).

The individual cells in some cases are closely packed among the surrounding lymphoid elements; in a large number of instances, however, they appear to lie in a limited clear space (Pl. II., Fig. 4, 6). This appearance may possibly be an artificial one arising as the result of shrinking during the course of preparation.

The number of cells visible in individual sections and in different parts of the same section varies considerably. In some sections as many as eighty or even more may be visible at once in a single field under a power of one hundred and forty diameters. In others they are present in varying but smaller numbers, and, in almost any, entire fields may in certain places fail to show any at all. This failure may in many instances be due to imperfect success in staining, but allowing this, there can be no doubt that the numbers present in different parts of the tumour vary greatly.

It is only quite exceptionally that any are present in the epidermal stratum. In one or two cases, however, I have detected specimens in the substance of interpapillary processes in sites which they could not have reached by accidental transfer during the course of the preparation of the sections. The continuous stratum of granulation-tissue beneath the papillary layer is the site in which they occur in greatest numbers, but specimens are also frequently present in considerable numbers within the papillary eminences. It is in the central, thickest portion of the continuous stratum that they occur in greatest numbers, and in passing outwards laterally from this they tend to appear in progressively smaller numbers, until towards the edges entire fields apparently devoid of them may be encountered. In those sites where traces of hair-follicles remain recognisable they are often present in specially large numbers (Plate II., Fig. 8).

In the portion of the tumour next beneath this, where the neoplasm is no longer continuous but is interposed in irregular masses between persisting por-

tions of normal dermal tissue, numerous specimens are also present. Here also as in the superior stratum they frequently are specially conspicuous in the neighbourhood of altered hair-follicles. The large masses of neoplasm around the sweat-glands and larger vascular trunks, too, often contain an abundance of them, while smaller numbers or isolated specimens are present in many of the masses of neoplasm generally distributed throughout the entire system of interstitial spaces. Here, as in the upper stratum, it is in the central portions of the tumour that they occur in largest numbers.

Their distribution is not, however, limited to the epidermal and dermal strata, for on passing downwards to the subcutaneous tissues scattered specimens may be found even in the very deepest parts. The numbers actually present in different sites varies in proportion to the degree to which these have become the sites of invasion by masses of lymphoid elements, but even where this has taken place only to a very limited extent, isolated specimens may be encountered (Plate I., Figs. 6, 7). For example, the specimens shown in Plate I., Figs. 4, 5 ; Plate II., Fig. 1, are situated in one of the interstitial spaces between the cells in fat-lobules in which an accumulation of lymphoid elements has only just begun to occur.

There can, then, be no doubt that in this specimen of "Delhi Boil" these peculiar bodies are generally distributed throughout the entire extent of the morbid tissue, and that the numbers of them present in particular areas correspond generally with the degree to which the morbid process has advanced there.

It remains now to consider the question of the nature of these bodies and of the relation which they may be regarded as holding to the disease.

Unfortunately it is as yet impossible to come to definite conclusions on either of these points. The cells have as yet only been observed in preserved tissues, and without definite data regarding their development and life-history generally the utmost that can be done is to indicate probabilities regarding their nature. After very careful consideration of the features presented by all the various forms present in the tissues, I am inclined to regard them as representing various stages of some simple organism of Mycetozoic nature. In the most recent systematic treatise dealing with the Mycetozoa or Myxomycetes—Zopf's "Schleimpilze" they are subdivided into Monadinæ and Eumycetozoa, and it is to the former group that the organism here dealt with appears to me probably to belong. The appearances presented by the various forms are, according to this view, to be regarded as corresponding to various stages of the development, and specially of the development of the Zoocysts or Sporocysts, of some Monadinic organism. Comparing the characters of the various specimens with one another, we have apparently to deal with the development of parent plasmodia or amœbæ which multiply by division, and in which sporoid bodies are gradually developed, the process terminating in some cases with the formation of one great spore, in others with that of a dense aggregate of smaller

ones. As has been just indicated, however, we are here dealing with probabilities only, and any definite conclusions can only be looked for after the organisms have been studied while alive, and the various stages in their life-history actually followed out in cultivations in suitable media.

The necessity for such observations is even more urgent in order to the attainment of any definite answer to the question of the relation which they bear to the occurrence of the disease with which they were in this case associated. There are, however, certain points rendering it possible, if not even probable that they actually were the specific causes of the disease. In the first place, there can in regard to this specimen be no question of accidental entrance of the parasites into the morbid tissues due to abnormal facilities presented by the surface, for there was no ulcerated, raw surface present, and, on the contrary, the horny layer of the epidermis was abnormally thickened and resistent. This in itself is in favour of a belief in their causal relation, as it is hard to see why such bodies should specially attain access to the diseased rather than to the healthy tissues unless some special structural facilities are provided in the former for their doing so. The general features of the distribution of the parasites also points in the same direction, for, as has been pointed above, they are found to occur in numbers varying in fairly close correspondence with the degree to which morbid changes have taken place. Much more significant, however, is the fact of their frequent presence in sites where such changes are only beginning to manifest themselves—in sites where small, isolated aggregations of the neoplasm are interpolated among wide areas of normal tissue elements.

The circumstances under which the disease generally tends to occur—its markedly endemic character, and specially its connection with the employment of certain sources of water supply—are also strongly in favour of the causative agency of bodies of the nature which those in the present case apparently possess, as the plasmodia and zoospores of the Monadinæ are specially likely to be present in water of special localities and of particular characters. The distribution of specially large numbers of the parasites in special relation to the remains of hair-follicles and in the neighbourhood of sweat-ducts is also of some importance as it seems possibly to indicate these structures as special channels of access to the substance of the tissues, and naturally they can only serve as such so long as they retain their normal characters and have not been pressed upon and obliterated by surrounding accumulations of neoplastic elements. The fact that the parasites occur in such deeply-seated areas as the subcutaneous fat-lobules is quite in accordance with the clinical experience that no superficial application of remedies is effectual in putting a stop to the disease, and that free excision or penetrating destruction of the tissue of the diseased area is necessary in order to do so.

It may, moreover, be pointed out that there are well-ascertained instances in which parasites of monadinic nature serve as the efficient cause of the de-

velopment of tumours in the host body which they affect, not merely as the result of accumulation of their own proper elements, but due to the occurrence of coincident processes of excessive hypertrophy in the host-tissues apparently of irritative origin. For example, in the case of Woronin's Plasmodiophora Brassicæ we have to deal with a parasite of this nature affecting various species of Brassica and causing both great hypertrophy of individual cells and accelerated rate in processes of cell division in the host.

The data which have been attained as the result of this examination of a specimen of typical Delhi Boil appear, then, to me to show that the diseased processes in cases of so-called "Oriental Sore" may certainly be associated with and possibly caused by the presence of peculiar parasitic bodies. In regard to this, however, it appears to be quite possible that such bodies may constitute a real cause of such disease without necessarily constituting its only cause. The neoplasm in specimens of Delhi Sore, such as the present one, presents no specific characters. All the changes in the normal tissues may be reasonably ascribed to mere persistent local irritation of certain intensity, and it is quite conceivable that such irritation may be connected with different exciting causes in different instances. Even, therefore, should it eventually be demonstrated that all cases of such disease occurring in and around Delhi are invariably and causatively related to the presence of the parasitic organisms here described, it by no means necessarily follows that all similar forms of disease occurring in other localities should have an identical causation. It is quite conceivable that similar morbid changes may arise as the result of the presence of other forms of parasitic organisms, or may even be developed apart from parasitic agency of any kind whatever.

CALCUTTA;

The 1st October 1885.

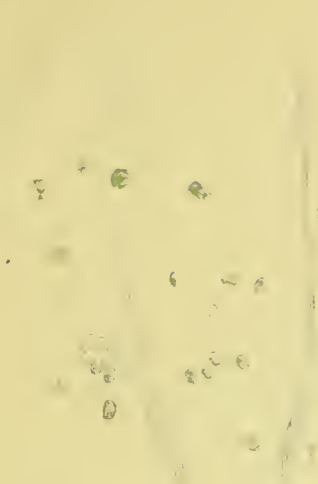


Fig. 1 × 122.

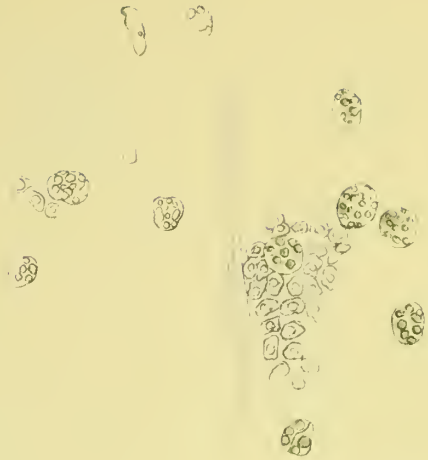


Fig. 2 ×. 340.

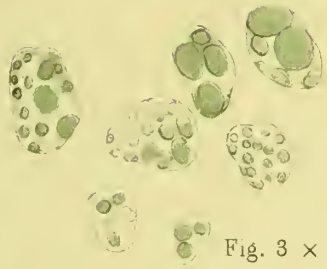


Fig. 3 × 680.

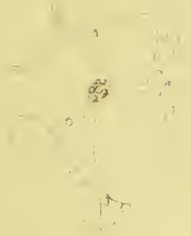


Fig. 4 × 122.

Fig. 5 × 680.

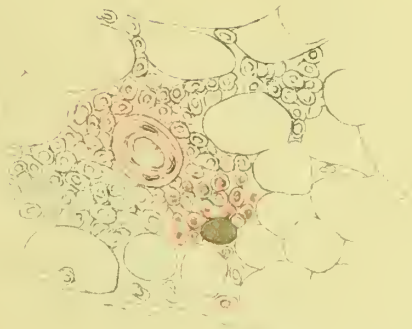


Fig. 6 × 340.

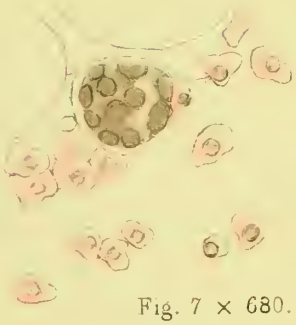


Fig. 7 × 680.

PARASITIC ORGANISMS IN A SPECIMEN OF DELHI-SORE.

Lithographed at the Survey of India Offices, Calcutta, September 1885

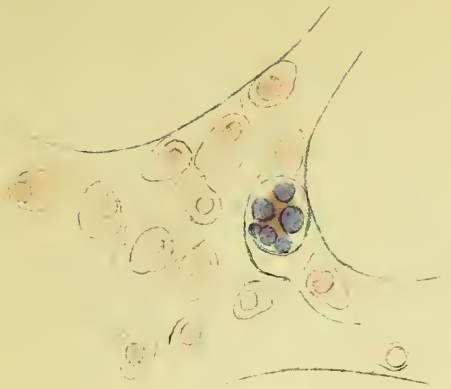


Fig. 1 × 680.

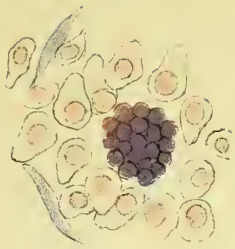
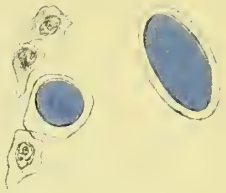


Fig. 2 × 680.



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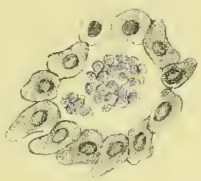

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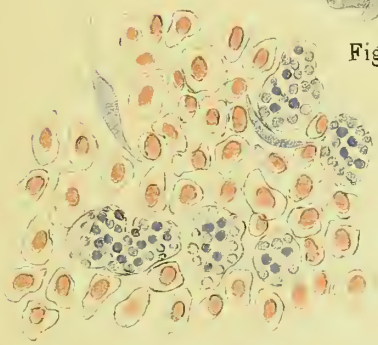


Fig. 5 × 680.

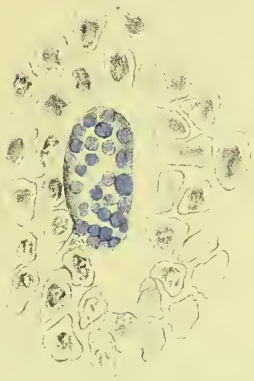


Fig. 6 × 680.

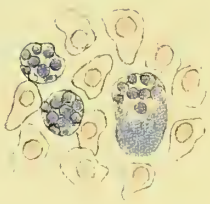


Fig. 7 × 680.

Fig. 8 × 122.

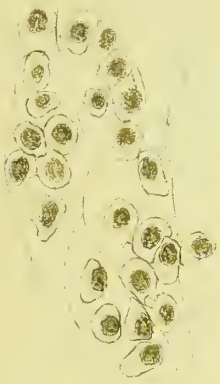
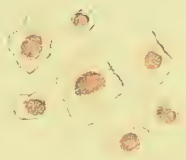


Fig. 12 × 680.



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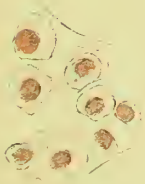

Fig. 14 × 680.

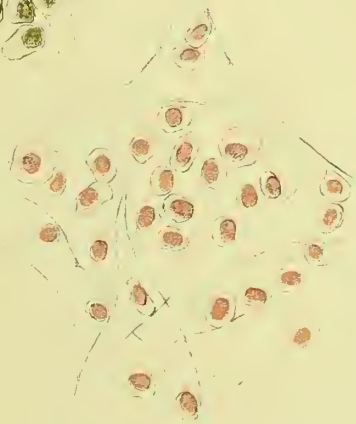

Fig. 13 × 680.

Fig. 11 × 680.

Fig. 10 × 680.

PARASITIC ORGANISMS AND TISSUE ELEMENTS IN A SPECIMEN OF DELHI-SORE.

Lithographed at the Survey of India Offices, Calcutta, December 1885.

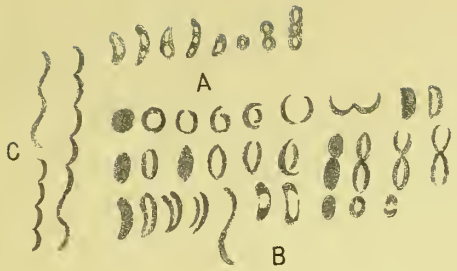

Fig. 1.

Fig. 3 × 37.

Fig. 2 × 6·5.

Fig. 4 × 37.

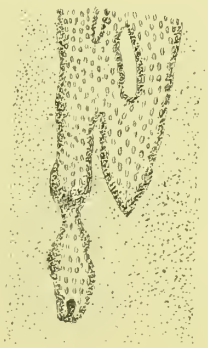

Fig. 6 × 37.

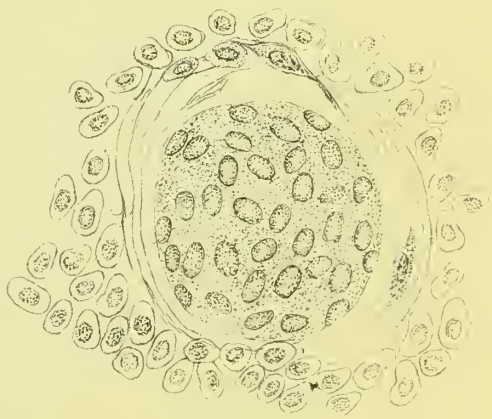

Fig. 5 × 680.

TISSUES IN A SPECIMEN OF DELHI-SORE, &c.

Lithographed at the Survey of India Offices, Calcutta, February 1886.